Comments to Parexel suggestions

Unfortunately I cannot comment on Metaprobe™ biomarkers since I have not managed to find any information on their use. However, the following comments may be relevant:

Liver function

Cytochrome P4501A2, the principal liver enzyme involved in aromatic amine N-oxidation, exhibits a wide range of interindividual variation for which various phenotyping methods using the probe drug caffeine exist [1]. These methods include the determination of various caffeine metabolite ratios in saliva [2,3] and urine [3-5], and caffeine-derived labeled CO in exhaled breath [6]. Dietary caffeine intake [2], smoking [2,4,5,7,8], gender [8], use of oral contraceptives [2] and chronic alcohol consumption [7] are associated with increased caffeine clearance (i.e., CYP1A2 induction). Furthermore, a C→A point mutation polymorphism in intron 1 of the CYP1A2 gene appears to confir high inducibility [3]. Thus, a gene-environment interaction may be a significant cause of the reported induction of CYP1A2 activity.

Contrary to the suggestion by Parexel, this test should not be used to identify 'closet smokers' since it lacks both sensitivity and specificity, and the study already includes determination of nicotine metabolites which will identify misreporting of smoking status.

Immune capacity

I could not find any information on the Tylenol challenge test or on other breath tests for measuring oxidative stress.

Mitochondrial capacity

The suggested assessment of mitochondrial capacity [9] uses administration of labeled ketoisocaproate and 2 hours post-administration determination of the decarboxylated analogue in exhaled breath. Studies of this kind normally use sequestered subjects and are therefore not ideally suited for the TE study. Since the effect of smoking has not previously been investigated it might be premature to use this type of breath test in the TE study.

Vascular epithelial health

Nitric oxide production, a non-specific marker of inflammatory response, can be measured in exhaled breath [10]. Although exhaled NO is easy to determine using a nitric oxide monitor, standardization of exhaled breath collection is difficult. Alternative methods for indirect determination of NO have focused mainly on measurement of NO metabolites (nitrate and nitrite) in induced sputum supernatant [11,14] and blood plasma [11]; however, these methods are not specific. Other methods, which are also not specific, include the determination of NO-related amino acids L-arginine and L-citrulline, and the intermediate Nϖ-hydroxy-L-arginine in plasma [13].

Other metaprobes

I think these are outside our field of interest.

Technical suggestions:

3-Ethenyl-pyridines

3-Ethenylpyridine is a useful marker for indoor air ETS gas phase monitoring. It cannot be used as a biomarker of smoke exposure since its metabolism in man (and experimental animals) has not been established. By analogy to other compounds of similar structure it would be predicted to undergo epoxidation in the side chain and hydrolysis of the epoxide to the diol. However, a number of compounds in tobacco smoke with a pyridine moiety and an alkyl group at the 3 position could undergo biotransformation to give similar products. Therefore, 3-ethenylpyridine cannot be recommended as a potential biomarker of tobacco smoke exposure.

Hb adducts of 3- and 4-aminobiphenyl

The hemoglobin adducts of 3-aminobiphenyl and 4-aminobiphenyl have been extensively reported as suitable biomarkers of exposure in both smokers and nonsmokers [14-17]. 4-Aminobiphenyl hemoglobin adduct levels fall on smoking cessation with an estimated half-life of 7-9 weeks, consistent with the lifetime of hemoglobin [17]. However, two technical limitations have to be taken into consideration: (1) 4-aminobiphenyl hemoglobin adduct levels differ significantly between slow and rapid acetylators [14,16], and (2) 4-aminobiphenyl DNA adduct levels in human peripheral lung do not correlate with smoking status [18].

Nicotine and nicotine metabolites

Determination of nicotine, cotinine, 3'-hydroxycotinine and their respective glucuroinde conjugates in 24-h urine samples will give a better estimation of nicotine exposure than determination of nicotine, cotinine and 3'-hydroxycotinine in spot blood samples. The presence of glucuronide conjugates of nicotine and its metaboilites have not been reported in blood.

Thromboxane B2 and Fibrinogen

This is something where we do need some external advice on suitable biomarkers of effect.

Isoprostanes

Determination of isoprostanes as biomarkers of effect has on numerous occasions been recommended by Benowitz; however, I have yet to see any data from Benowitz on such studies. I agree with Parexel that a single isoprostane probably needs to be considered, and am currently in the process of reviewing the literature to identify the most suitable candidate biomarker(s). The F2-isoprostane family consists of a series of chemically stable prostaglandin F2 derivatives formed by peroxidation of arachidonic acid in phospholipids. Consequently, these compounds are suggested to represent a reliable index of lipid peroxidation (oxidative stress) in vivo [19]. However, analysis is both time consuming and complex. Recently a simplified method was reported using solid-phase extraction columns, high-performance liquid chromatography, and stable isotope dilution capillary gas chromatography/electron capture negative ionization mass spectrometry for 8-epi-PGF2α, one of the most abundant F2-isoprostanes in biological samples [20]. I cannot make any more specific comments until I complete review of the literature on smoking and isoprostane levels.

Isopentane is not generally determined in exhaled air; however, pentane in exhalate has been determined as an index of lipid peroxidation [21,22]. Although

hydrocarbon-based breath tests for determination of oxidative stress are noninvasive, several minor technical difficulties (e.g., standardized washout period, air contamination) need to be resolved [23]. Without a complete review of the literature (over 100 publications on pentane in exhaled breath) I cannot make any further comments. However, my initial impression is that real-time monitoring of exhalate is probably not an area of biomonitoring that we want to get involved in.

Bronchial cells

I agree with Parexel that collecting nasal lavage may be technically less difficult than collecting induced sputum. However, the question still remains as to what should be investigated in the collected samples. Clara cell protein-16 (CC-16), a biomarker suggested by HHO, can still be measured in nasal lavages [5], but I am unsure as to what additional information this marker will contribute to the TE study.

Collecting induced sputum has the advantage that previous molecular epidemiology studies have investigated smoking-associated DNA adduct formation in bronchoalveolar macrophages [25,26]. However, it is unlikely that the required careful standardization of induced sputum collection using saline washing [27] was applied in these studies.

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